12-1-2001

Inheritance of Dorsal Fin Coloration in the Metriaclima Species Complex (Teleostei: Cichlidae) of Lake Malawi

Paulette Reneau
Western Kentucky University

INHERITANCE OF DORSAL FIN COLORATION IN THE *METRIACLIMA* SPECIES COMPLEX (TELEOSTEI: CICHLIDAE) OF LAKE MALAWI

A Thesis

Presented to

The Faculty of the Department of Biology

Western Kentucky University

Bowling Green, Kentucky

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

By

Paulette Clarke Reneau

December 2001

INHERITANCE OF DORSAL FIN COLORATION IN THE *METRIACLIMA* SPECIES COMPLEX OF LAKE MALAWI (TELEOSTEI: CICHLIDAE)

Date Recommended 23 NOV 2001

Director of Thesis

Elmer Gray 1-11-02

Director of Graduate Studies Date

ACKNOWLEDGMENTS

This research was completed with the combined effort of many people. I would like to thank Old World Exotics, Homestead, Florida for care and shipping of the cichlids used in the research, and Stuart Grant of Salima, Malawi for collecting and exporting them for use. Many thanks are also extended to Peter Smith and Dr. Irv Kornfield for sharing unpublished data from their microsatellite DNA analysis of population structure in the *Metriaclima zebra* complex.

I would like to express my gratitude and appreciation to the following faculty members in the Biology Department. My most heartfelt "Thank you" is extended to Dr. Douglas McElroy, my major professor, who has been a source of encouragement and immeasurable instruction. Your patience, academic guidance and unfailing quest for the deeper truth in any statement has helped me keep a clear focus and solve research problems. My gratitude is also extended to Dr. Robert Wyatt for his help with all the figures. Thanks to Dr. Lawrence Alice and Dr. Bonnie J. Furman for serving as members of my committee and for providing helpful suggestions on my research and thesis.

Thanks to the faculty, staff, and graduate students of the Department of Biology at Western Kentucky University for encouragement, friendship, and support. Your presence made my stay an enjoyable and rewarding experience. Thanks also to the Graduate School at Western Kentucky University for providing funding throughout my academic career. Partial

support for this research was provided by a grant from the National Science Foundation (DEB97-075232) to Irv Kornfield.

I would like to thank my parents, Mr. Orton Clarke and Mrs. Elaine Clarke, for continued love, support, and encouragement. Finally, to my husband and best friend, Franz, "Thanks for your love, patience, and relentless optimism leading to the completion of this thesis."

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INHERITANCE OF DORSAL FIN COLORATION IN THE METRIACLIMA SPECIES COMPLEX (TELEOSTEI: CICHLIDAE) OF LAKE MALAWI

Name: Paulette Clarke Reneau Date: December 2001 Pages 33

Directed by: Douglas M. McElroy, Lawrence A. Alice, and Bonnie J. Furman

Department of Biology Western Kentucky University

The rock-dwelling cichlids (mbuna) of Lake Malawi have undergone an explosive evolution, giving rise to an assemblage of 300-500 species within the last one million years. Most widespread mbuna 'species' are characterized by the presence of local endemic populations, differing primarily in coloration and often of uncertain taxonomic rank. The recency and rapidity of speciation within the mbuna have led to difficulties in reconstructing an accurate species-level phylogeny, in turn limiting our ability to elucidate the evolutionary dynamics associated with divergence of coloration and other characters. Based on morphometric and meristic characters, Stauffer *et al.* (1997) erected a new genus along with ten new species. Here we use classical Mendelian analyses to investigate the inheritance and evolution of red dorsal fin coloration in *Metriaclima thapsinogen* and *M. emmiltos*, phenotypically similar taxa endemic to habitats separated by at least 350 km. Multiple crosses involving single males and four to five females were established in the laboratory. Crosses resulted in five F_1 broods and four F_2 broods hybrid progeny (15-25 fry each), in addition to broods from

control crosses. F_1 and F_2 hybrid individuals were assessed for dorsal fin coloration while parental and F_1 hybrid individuals were evaluated, additionally, for eight meristic characters. Phenetic relationships among geographically similar morphs were assessed and compared to Stauffer *et al.* (1997) findings.

Upon maturity, all hybrids showed red dorsal fin coloration. None of the eight meristic variables showed significant differences in means among parental, F_1 and control hybrid groups. Two meristic variables displayed significant fluctuating asymmetry in F_1 hybrids relative to parental and control groups, but this degree of asymmetry was less than predicted under a null model. UPGMA clustering resulted in *M. thapsinogen* and *M. emmiltos* grouping closer together than geographically proximate *M. zebra*. These data suggest the genetic basis for red dorsal fin coloration is allelic between *M. thapsinogen* and *M. emmiltos*, and are consistent with their evolution from a common red dorsal ancestor followed by lake-wide dispersal. Alternative scenarios, including the existence of color and regulatory loci in each population and differential expression of a set of ancestral color polymorphisms are consistent with our findings.

INTRODUCTION

The cichlid fish faunas of the East African Lakes of Malawi, Tanganyika, and Victoria have undergone an explosive evolution, giving rise to species assemblages consisting of hundreds of species in each lake. Lake Malawi alone is believed to contain as many as 1000 species of cichlids (Lewis *et al.* 1986; Turner 1996), more than any other lake in the world. About 99% of these species have been described as endemic (Ribbink *et al.*, 1983a; Konings, 1990; Greenwood, 1991; Ribbink, 1991), originating from a single common ancestor within the past 1 - 2 million years (Fryer and Iles, 1972; Ribbink *et al.*, 1983a; Owen *et al.*, 1990). The enormous species diversity present in Lake Malawi raises a myriad of questions as to the mechanisms involved in speciation that could have brought about the existence of such an evolutionarily unique fauna (Grant, 1986).

Of the over 1000 species of fishes that inhabit Lake Malawi, there is a group of about 500 species that occupy the rocky habitats around the lake that fall into about a dozen genera (Eccles and Trewavas, 1989). These fishes, collectively referred to by their Chitonga name, the mbuna, meaning rock-dwelling (Trewavas, 1935; Ribbink *et al.*, 1983a; Reinthal, 1987), are noted for their bright coloration. Mbuna are relatively small, being less than 100 mm standard length (SL) when fully matured. Others range from 150 mm SL to less than 40 mm SL (Ribbink *et al.*, 1983a). Males possess a distinctive blue body with black vertical bars along the flank in contrast to the drab coloration of females. Due to the bright coloration that exists within populations, studies (e.g., Deutsch, 1997; McElroy *et al.*, 1991) have shown

that color is an important characteristic in the mbuna species, specifically in mate selection. Males establish territories in the rocky regions, build nests and may remain at one site for years. Females swim above these nest sites and when ready to spawn choose a mate; males solicit partners by carrying out courtship displays. Females deposit eggs on the rocks while males swim over them, in the process fertilizing the eggs. Females then place the fertilized eggs into their mouths for incubation until fry develop and are relatively independent. With the importance of establishing territories along rocky patches, space becomes an extremely critical variable in Lake Malawi.

Lake Malawi is the third largest of the African Great Lakes and lies at the southern end of the East African Rift System. Its position just below the Equator (9°30 S to 14°30 S) leads to relatively constant water conditions, with limited seasonality; however, climatic changes on a global scale can cause major lake-level fluctuations, creating cycles of habitat destruction and creation (Fryer 1959; Ribbink *et al.* 1983a; McKaye and Gray 1984).

These lake-level shifts may have significant implications for differentiation of taxa by microallopatry (Owen *et al.*, 1990). Fryer and Iles (1972) propose that lake-level fluctuations following climatic variation would influence spatial distribution of the fishes in the lake and create opportunities for allopatric speciation. With a drop in lake levels, Fryer (1977) determined that populations might be forced to vacate areas that were becoming unsuitable. Evidence propound that lake levels were 200 - 300 m below the present shoreline about 28 - 40 thousand years ago; even more recently (6 -10 thousand years ago) lake levels

were 100 - 450 m below present (Crossley *et al.*, 1984). Bottom topography and seismic data (Scholz and Rosendahl, 1988) conclude that rocky habitats currently supporting hundreds of species did not exist during these low lake level stands, and refugia for endemics were absent as recently as 10 kya. The implication is that much of the current diversity in Lake Malawi must have been generated since the last desiccation (Owen *et al.,* 1990).

Lake level fluctuations might be especially significant for the mbuna, a monophyletic group of at least 300 species that inhabit rocky habitats patchily distributed around the periphery of the lake to a depth of up to 50 m (Trewavas, 1935; Ribbink *et al.*, 1983a; Reinthal, 1987). While some species have lake-wide distributions, many others are restricted to one or a few areas; endemism within zones of the lake exceeds 90% (Ribbink, 1991). These fishes comprise speciose and ecologically diverse communities (Hori, 1993; Stauffer, 1994) that include a number of species complexes whose members are extremely closely related (Kornfield, 1978; Meyer *et al.*, 1990; Moran and Kornfield, 1993; Kornfield and Parker, 1997). A lack of morphological synapomorphies (Stiassny, 1991) and the presence of shared ancestral polymorphisms at the molecular level (Kornfield, 1978; Moran and Kornfield, 1993) have confounded reconstruction of mbuna phylogeny (Kornfield and Parker, 1997; Mayer *et al.*, 1998; Albertson *et al.*, 1999).

Most individual species display a wide range of color diversity among isolated populations (Ribbink *et al.*, 1983a; Eccles and Trewavas, 1989); there is also increasing evidence of within-population variability (Kornfield, unpubl. data). This color diversity is particularly

evident in the *Metriaclima zebra* (Boulenger) complex (Stauffer *et al.*, 1997). In addition to a blue-black (BB) form (*M. zebra*) distributed lake-wide, there also exist isolated populations displaying alternative phenotypes including blue, gold, black dorsal, and red dorsal (each with several representative forms). Ecological and behavioral studies have shown that color is an important communication and species recognition characteristic in the mbuna and other cichlids (Houde and Endler 1990; McElroy *et al.*, 1991; Guthrie and Muntz, 1993; Deutsch, 1997; Seehausen and van Alphen, 1998) leading many to classify populations differing in coloration alone as separate species (e.g., Ribbink *et al.*, 1983a; Stauffer *et al.*, 1997).

In an effort to more accurately describe members of the mbuna group, Stauffer *et al.* (1997) revised the *Pseudotropheus zebra* (Boulenger) species complex, erecting a new genus (*Metriaclima)* and 10 new species on the basis of morphometric and meristic characters. The red dorsal phenotype occurs in six populations within the *M. zebra* complex that have a disjunct distribution throughout Lake Malawi. Stauffer *et al.* (1997) classified each of these populations as separate species; however, the evolutionary origin of these populations remains unresolved. Stauffer *et al.* (1997) suggested that red dorsal lineages are sister to geographically proximate BB populations; under this view, the red dorsal phenotype would be the result of convergent or parallel evolution in the various taxa. An alternative hypothesis would be that the red dorsal taxa may themselves constitute a monophyletic group, implying a single origin and common genetic basis of the red dorsal phenotype. Finally, red dorsal populations may be the result of recurrent expression of common ancestral

color polymorphisms, as suggested by Seehausen *et al.* (1999) for Lake Victoria cichlids. The lack of a resolved phylogeny prevents evaluation of these alternative hypotheses using a strict comparative approach.

Here we employ classical Mendelian genetic analysis to test for complementation in dorsal fin coloration between two phenotypically similar red dorsal species, *Metriaclima thapsinogen* (Stauffer, Bowers, Kellogg, McKaye) and *M. emmiltos* (Stauffer, Bowers, Kellogg, McKaye), endemic to southern and northern Lake Malawi, respectively. The preliminary results of the complementation test between two red dorsal lineages will be used to investigate two mutually exclusive evolutionary hypotheses: (1) Red dorsal fin coloration in *M. thapsinogen* and *M. emmiltos* is a result of common ancestry. If hybrid lineages breed true for the red phenotype, the indication would be that the genetic basis for that trait in the two populations is the same, i.e., allelic, which in turn suggests a single evolutionary origin; and (2) Existence of the red phenotype in isolated populations may result from convergent evolution. Segregation of blue phenotypes in hybrid lineages would indicate that the trait is controlled by different genes in different populations, i.e., non-allelic, and thus suggests multiple evolutionary origins of the red dorsal phenotype.

We examine inheritance of dorsal fin phenotype in F_1 and F_2 hybrids; in addition, we test for evidence of developmental incompatibility between *M. thapsinogen* and *M. emmiltos* through analysis of phenotypic variance and fluctuating lateral asymmetry in hybrids relative to control crosses. We relate our findings to the data of Stauffer *et al.* (1997), and discuss their

implications for understanding the evolutionary history of red dorsal lineages within the *Metriaclima zebra* complex.

MATERIALS AND METHODS

Specimen Acquisition and Care

Parental individuals were wild-caught fish collected by registered exporters from Lake Malawi in November 1998. *Metriaclima thapsinogen* individuals were collected from Eccles Reef, 14°22'S, 35°28'E, in the southeast arm of Lake Malawi (Fig. 1). *Metriaclima emmiltos* individuals were collected from Chitande Rocks near Chilumba 10° 28′S, 34° 12'E, along the northwest coast of the lake (Fig.1). These populations are separated by ~350 km. Additionally, two sample groups of *Metriaclima zebra* (BB) representing both northern and southern regions of Lake Malawi were collected from Nkhata Bay 11° 33'S, 34° 18'E and Namalenje, 14° 6'S, 35° 11'E respectively (Fig. 1). All fish were shipped to Western Kentucky University, where they were housed in glass aquaria and maintained at 27° C under an Light : Dark 16:8 cycle. Fish were fed commercial flake food daily and subjected to partial water changes weekly to decrease nitrate levels and to induce spawning.

Production and Phenotypic Characterization of Hybrids

Multiple crosses between *M. thapsinogen* (Mt) and *M. emmiltos* (Me) were established in 57 L and 76 L glass aquaria, each consisting of a single male and four to five females. Five to ten sets of each of the reciprocal crosses were established. Control crosses of *M. thapsinogen* (Mt) **x** *M. thapsinogen* (Mt) and *M. emmiltos* (Me) **x** *M. emmiltos* (Me) were

established in a similar manner. Females were checked daily for the presence of eggs in the mouth, indicating that spawning had occurred. Brooding females were captured during their second week of egg incubation and isolated until their fry were released from the mouth.

F_1 progeny resulting from *M. thapsinogen* (Mt) **x** *M. emmiltos* (Me) and control crosses were grown to reproductive maturity under conditions similar to those described above. F_1 **x** F_1 crosses were established from the resultant pool of MtMe hybrids; these crosses were maintained and F_2 progeny treated as described above. Once adult coloration was evident at approximately 30 mm standard length, $F_1$and F_2 progeny were sexed and characterized for dorsal fin phenotype.

Meristic Analysis of Parentals and Hybrids

Parental, F_1 hybrid, and control individuals were used in an indirect test of developmental incompatibility in the hybrids. Samples of both parental taxa and all hybrid individuals having a body length greater than 35 mm were assessed for eight meristic variables (Fig 2). Three of these meristic variables (pectoral fin rays, flank bars, pelvic fin rays) were recorded bilaterally for analysis of fluctuating lateral asymmetry, measured as the difference (D_x) in number of meristic elements on the left (X_L) and right (X_R) sides of the body ($D_x = X_L - X_R$). The number of pelvic fin rays showed no variation within or among groups, and therefore was excluded from analysis.

Parental, control and hybrid groups were tested for significant differences in means for midline variables (dorsal spinous rays, dorsal soft rays, anal fin rays, upper tooth rows, lower tooth rows, pectoral fin rays, flank bars, pelvic fin rays) using Analysis of Variance (ANOVA). In addition, the five midline variables were qualitatively examined for evidence of increased standard deviation in hybrids relative to control and parental groups. Bilateral characters were tested for the presence of significant fluctuating lateral asymmetry by comparing estimates of kurtosis among groups using ANOVA.

Phenetic Relationships of Red Dorsal and BB Populations

Phenetic similarity between red dorsal *M. thapsinogen* and *M. emmiltos* was assessed relative to geographically proximate BB populations. Meristic data described above were collected from northern *M. emmiltos*, southern *M. thapsinogen*, northern *M. zebra* and southern *M. zebra*. The means of these data were analyzed using principal components analysis (PCA) of the resulting correlation matrix. PCA was utilized to compute uncorrelated axes so that factor scores could be generated to to calculate Euclidean distances between the means of all populations; these distances were clustered using UPGMA.

Results from this analysis were compared to a reanalysis of data taken from Stauffer *et al.* (1997) for five groups of *Metriaclima,* from Eccles Reef (*M. thapsinogen*), Chilumba (*M. emmiltos*), Namalenje (*M. zebra*), Nkhata Bay (*M. zebra*), and Chitande Rocks (*M. zebra).* Principal component analyses of the correlation matrix were carried out using sample means

for 24 morphometric and nine meristic characters. Five additional meristic variables (lateral-line scales, dorsal fin rays, anal fin spines, pelvic fin rays, and gill raker on first epibranchial) recorded by Stauffer et al. (1997) showed no variation among these taxa, and were excluded from analysis. Morphometric data were size-corrected using the approach of Burnaby (1966) in NTSYS-pc (Rohlf, 2000) prior to analysis. Except where noted, all statistical analyses were done using SYSTAT 9.0 (SPSS Inc., 1999).

Separate PCAs were conducted for morphometric and meristic data. The first factor of the size-corrected morphometric data was plotted against the first factor of the meristic data. Euclidean distances between each of the five groups were calculated from factor scores and clustered using UPGMA. A second UPGMA cluster analysis was conducted on Euclidean distances calculated using meristic data only; this analysis was designed to maximize the similarity between the Stauffer *et al.* (1997) data set and that derived from our measurements of parental taxa. Nevertheless, Stauffer *et al* (1997) meristic data did not include measures of bilateral symmetry in pectoral fin rays and flank bars (as all counts were taken from only one side of the fish), while our data set did not include scale and gill raker counts.

RESULTS

Five broods of *M. thapsinogen* x *M. emmiltos* (MtMe) F_1 hybrids were produced; all broods were derived from *M. thapsinogen* (♀) x *M. emmiltos* (♂) crosses. In addition, single broods of each of the control crosses (*M. thapsinogen* x *M. thapsinogen* and *M. emmiltos* x *M. emmiltos*) were produced. Initial brood sizes of F1 progeny were 15-25, consistent with the expected fecundity of *Metriaclima* species (Fryer and Iles, 1972). Four different males fathered the five MtMe F_1 broods; all broods were produced by different females. Four MtMe F_2 broods were produced, each derived from a different set of parents; as with F_1 progeny, brood sizes ranged from 15 - 25 (Table 1).

Hybrid progeny from both control crosses (total N=33) displayed the red dorsal fin phenotypes, as expected. All F_1 hybrid individuals (N=90) also displayed red dorsal fins; there was no indication of segregation or of phenotypes intermediate between red and blue. All F_2 hybrids (N=68) also displayed red dorsal fins (Table 1).

None of the eight meristic variables showed significant differences in means (as indicated by overlapping standard errors) among parental, F_1 control and hybrid groups (Table 1). In addition, there was no indication of increased variability in hybrids relative to either parentals or controls; standard deviations showed no qualitative differences among groups (Table 2). However, two of three bilateral characters showed significant kurtosis in the hybrid group relative to both parental and control groups (Table 3); both number of pectoral fin rays and

number of flank bars were strongly leptokurtic (t=30.96 and 8.43, respectively; df = 26 and $p < 0.0001$ for both). Progeny from control crosses showed no asymmetry for any bilateral characters, and less qualitative variability in hard dorsal fin rays and soft dorsal fin rays than both parental and hybrid groups (Table 3).

Principal components analysis of meristic data from parental, control and hybrid F_1 groups resulted in three factors that carried a significant amount of variation (87% of the total variation in the data set) (Table 4). UPGMA clustering of population centroids grouped *M. emmiltos* and *M. thapsinogen* together to the exclusion of both northern and southern *M. zebra*, which also formed a distinct cluster (Fig. 3).

UPGMA clustering of five populations within the *M. zebra* group derived from reanalysis of Stauffer *et al.*'s (1997) data produced somewhat different results. Factor scores generated from morphometric and meristic data resulted in *Metriaclima zebra* from Nkhata Bay clustering tightly with *M. emmiltos* (both from northern Lake Malawi), while *M. zebra* from both Namalenje (southern Lake Malawi) and Chitande (northern Lake Malawi) clustered with *M. thapsinogen* from the south (Fig.4). When clustered using factor scores from meristic data alone, *M. zebra* from Namalenje and *M. zebra* from Chitande once again clustered tightly. *M. zebra* from Nkhata Bay and *M. emmiltos* formed a separate cluster, while *M. thapsinogen* joined outside of both clusters (Fig 5).

DISCUSSION

Genetics of Coloration

Lack of segregation of alternative phenotypes in F_1 and F_2 hybrids indicates that the genetic basis for the red dorsal phenotype is allelic between *Metriaclima thapsinogen* and *M. emmiltos.* Despite a relatively small F_2 sample size (N=68) we can reject the idea that the lack of observed complementation is due to chance alone. Genetic models hypothesizing complementation fail to predict the observed distribution of phenotypes in the F_2 sample. For example, assume that red and blue phenotypes are coded for by alternative alleles at a given color locus and that color evolved independently at different loci for each of these two species. Thus, if the red allele is dominant over blue, and red coloration is controlled by a dominant allele at alternative loci in *M. thapsinogen* and *M. emmiltos* (i.e. A and B), we would predict that 1/16 of F2 individuals should display the blue phenotype (i.e., bearing the aabb genotype). The binomial probability of seeing all red individuals in a sample of 68 under this model is $p = 0.0124$; thus, we can reject the hypothesis that this complementation model is consistent with the data. All other complementation models (e.g., where red is recessive, epistasis, etc.) would predict an even higher proportion of blue individuals and therefore be even less likely.

Earlier studies (Kornfield, 1991; Seehausen *et al.*, 1999) have suggested that coloration in cichlids involves the interaction of both color and regulatory loci. Kornfield (1991) proposed

a model for red dorsal fin coloration in the *M. zebra* group that consisted of a color locus with two alleles, with blue dominant over red, and a color-regulatory locus with two dominant/recessive alleles. Under this model, expression of the color locus is dependent on expression at the regulatory locus, such that blue individuals can arise when they carry and express a blue genotype or are homozygous at the regulatory locus regardless of their genotype at the color locus. Seehausen *et al.* (1999) proposed a similar schematic model for Lake Victoria cichlids, suggesting the existence of two structural gene loci, each responsible for the color of the ventrum or dorsum, and a set of polymorphic regulatory loci. Here, different color combinations would result from differential expression of the various color loci.

Both of these models are consistent with our data. As *M. thapsinogen* and *M. emmiltos* populations breed true for the red dorsal phenotype, we can assume they are homozygous for expression of the regulatory locus (loci). Further, lack of segregation of alternative phenotypes indicates the color gene(s) are carried at the same locus (loci) in the two taxa. Crosses between red and blue species are required to estimate the actual or effective number of genetic factors involved.

Meristic Analysis

The degree of fluctuating lateral symmetry has been used to indicate developmental breakdown among genetically distinct lineages (Grobler *et al.*, 1999; Klingenberg and

Nijhout, 1999). In addition, phenotypic variability has often been studied in *Drosophila melanogaster* (e.g., Woods *et al.*, 1999) and other taxa to help determine the genetic mechanisms responsible for the varied effects on trait variability and developmental stability. Analyses of meristic data in *M. thapsinogen and M. emmiltos* suggest little overall genetic divergence between *M. thapsinogen* and *M. emmiltos*. None of the midline characters examined showed any evidence of increasing variability in F_1 hybrids relative to parental or F_1 control lines. While F_1 hybrids showed some asymmetry (in contrast to F_1 from control crosses), the degree of asymmetry was less than expected under a null model. One possible explanation for this reduced asymmetry is the fact that hybrids were produced under controlled aquarium conditions. The lack of any asymmetry in F_1 control groups is also consistent with this explanation.

UPGMA clustering of meristic characters indicated that red dorsal populations clustered together to the exclusion of northern and southern *M. zebra* populations. This grouping further supports the view that *M. thapsinogen* and *M. emmiltos* are genetically similar to one another relative to geographically proximate BB morphs. These findings are contrary to Stauffer *et al.* (1997) assertion that the red dorsal phenotypes are more closely related to BB from the same part of the lake than they are to one another (see also below). By contrast, these three lines of evidence suggest a close genetic relationship between red dorsal *M. thapsinogen* and *M. emmiltos*.

Population Relationships

Assuming allelism of the red dorsal phenotype, how can we account for the disjunct distribution pattern seen among red dorsal fin populations throughout Lake Malawi? Taken together, our data suggest that coloration in *M. thapsinogen* and *M. emmiltos* is of common origin. To the extent that these species are representative, this suggestion might lead us to hypothesize a common evolutionary origin of coloration in all red dorsal endemics in the *Metriaclima* species complex. Under this hypothesis, a single origin of the red dorsal phenotype would have been followed by lake wide, but noncontinuous, dispersal to generate the current distribution of red dorsal forms throughout Lake Malawi. Ongoing population genetic analyses of both red and blue dorsal *Metriaclima* populations (Smith and Kornfield, unpubl. data) corroborate this hypothesis; using microsatellite DNA loci, these authors have generated strong statistical evidence for monophyly of both red and blue dorsal lineages in four species across 10 marker loci (P. Smith, pers. comm).

Alternatively, the lack of complementation in dorsal fin coloration might result from inheritance and differential expression of a set of ancestral color genes in the two species. Numerous lineages, from different species complexes within Lake Malawi as well as the older, morphologically and ecologically more diverse lineages of Lake Tanganyika possess the red phenotype. This pattern suggests that coloration may be a pleisiomorphic trait in Lake Malawi, and may have arisen independently in Lake Malawi and Lake Tanganyika (which have never shared a basin connection [Scholz and Rosendahl, 1988]). Seehausen *et*

al. (1999) suggest the existence of ancient color genes as a possible mechanism to generate similar color polymorphisms among isolated populations. Under this model, recurrent expression of ancestral color genes silenced in the BB lineage, possibly linked to geographical isolation, could have occurred during the history of Lake Malawi to produce the observed distribution of red dorsal populations. Such a process would be consistent with our observed lack of complementation. However, under this model, we would not necessarily expect meristic and/or microsatellite DNA similarity among populations with similar phenotypes, as appears to be the case from our data and that of Smith and Kornfield (P. Smith, pers comm.)

Finally, parallel evolution of a derived red dorsal phenotype may be responsible for the distribution of red dorsal taxa. This model seems highly unlikely for two reasons. First, the independent evolution of the same genetic mutation in different lineages is unlikely. Second, this model implies that isolated red dorsal taxa would be sister to geographically proximate BB forms. As such, we might expect red and blue populations to cluster together on the basis of meristics, which they do not.

Systematic Implications

Based on a combination of morphometric and meristic characters, Stauffer *et al.* (1997) concluded that all populations in the *M. zebra* complex exhibiting the blue/black (BB) color pattern belong to a single species (*M. zebra*), despite some geographic differentiation. They

characterized each of the six red dorsal populations as distinct species. Our reanalysis of their data support this interpretation; geographically proximate red dorsal and BB populations clustered together to the exclusion of phenotypically similar populations from different regions on the basis of a combination of morphometric and meristic data. Clusters based on meristic data alone, while not presenting as clear a pattern, do not support a close phenetic relationship between red dorsal forms (to the exclusion of BB taxa).

Our meristic data, however, reveal a different pattern. In analysis of meristic data derived from parental taxa used in this study, red dorsal and BB populations form distinct clusters regardless of geography. The contrasting cluster pattern generated from Stauffer *et al.* (1997) data and our data can be attributed to differences in characters. Additionally, when PCAs were done on both data set different characters displayed higher loadings (Table 4, Table 5)

In combination with our genetic and fluctuating asymmetry data, we thus propose that there exist common meristic features that persist within the group, linking northern and southern populations. In addition, our data strongly support the interpretation that red dorsal fin coloration in both northern and southern populations is a result of common ancestry. More detailed populational analysis and broader sampling of taxa will be required to resolve this issue.

Table 1. Number of broods and individuals produced from crosses between *M. thapsinogen* (Mt), *M. emmiltos* (Me) and their F_1 hybrids (MtMe). Four of the five MtMe F_1, and all F_2 hybrid broods have different fathers. All broods have different mothers. All MtMe F_1 broods are derived from Mt_f x Me_m crosses.

Cross	$MtMtF_1$	$MeMeF_1$	$MtMeF_1$	$MtMeF_2$
Broods	1	1	5	4
Number of Fish	13	12	90	68

Table 2. Meristic values for samples of four *Metriaclima* populations. No significant differences exist among sample means for any character.

	Mode	Range	Mean±SE
***M. thapsinogen* (Eccles Reef) N=15**			
Dorsal-fin spines	17	14 - 19	16.733±0.267
Dorsal-fin rays	8	7 - 10	8.133±0.215
Anal fin	10	8 - 11	9.867±0.236
Pectoral fin rays (right)	13	12 - 14	12.733±0.182
Pectoral fin rays (left)	13	12 - 14	12.667±0.159
Teeth rows (lower jaw)	2	1 - 3	2.067±0.118
Teeth rows (upper jaw)	2	1 - 2	1.933±0.067
Vertical barring (right)	6	5 - 7	6.200±0.145
Vertical barring (left)	6	5 - 7	6.200±0.145
***M. emmiltos* (Chilumba) N=22**			
Dorsal-fin spines	17	17 - 19	17.238±0.117
Dorsal-fin rays	8	6 - 10	8.048±0.185
Anal fin	10	9 - 10	9.905±0.063
Pectoral fin rays (right)	12	11 - 13	11.952±0.139
Pectoral fin rays (left)	12	11 - 13	12.095±0.112
Teeth rows (lower jaw)	2	1 - 4	2.143±0.156
Teeth rows (upper jaw)	2	1 - 2	1.667±0.105
Vertical barring (right)	6	5 - 7	6.190±0.107
Vertical barring (left)	6	5 - 7	6.286±0.117
***M. zebra* (Nkhata Bay) N=11**			
Dorsal-fin spines	17	15 - 18	16.727±0.333
Dorsal-fin rays	7	6 - 10	7.818±0.400
Anal fin	10	9 - 11	9.909± 0.211
Pectoral fin rays (right)	11	8 - 13	11.455±0.340
Pectoral fin rays (left)	11	8 - 13	10.727±0.273
Teeth rows (lower jaw)	2	1 - 2	1.909±0.091
Teeth rows (upper jaw)	2	1 - 3	2.000±0.135
Vertical barring (right)	7	6 - 9	7.273±0.237
Vertical barring (left)	7	7 - 8	7 .09±0.163
***M. zebra* (Namaleje) N=14**			
Dorsal-fin spines	17	16 - 18	17.214±0.194

	Mode	Range	Mean± SE
Dorsal-fin rays	8	7 - 11	8.357±0.269
Anal fin	10	9 - 10	9.929±0.071
Pectoral fin rays (right)	12	9 - 14	12.000±0.363
Pectoral fin rays (left)	12	9 - 14	12.000±0.363
Teeth rows (lower jaw)	3	1 - 3	2.429±0.173
Teeth rows (upper jaw)	2	2 - 3	2.357±0.133
Vertical barring (right)	6	5 - 6	6.100±0.091
Vertical barring (left)	6	5 - 6	6.100± 0.091

Table 3. Phenotypic and meristic comparison of *M. thapsinogen* (Mt), *M. emmiltos* (Me), F_1 progeny from control (MtMt and MeMe) and hybrid crosses (MtMe). For F_1 groups, phenotypic characterizations are based on the total number of individuals produced (see Table 1); meristic results are derived from analysis of all individuals greater than 35 mm at the time of data collection. Note the relative uniformity of individuals from F_1 control groups, and presence of highly significant leptokurtosis (indicated by asterisks) in pectoral fin ray and flank bar symmetry in the F_1 hybrid group.

Character	Mt (N=15)	Me (N=22)	MtMt (N=7)	MeMe (N=5)	MtMe (N=27)
Phenotypic					
Dorsal Fin Color	Red	Red	Red	Red	Red
Midline (Mean ± SD)					
Dorsal	16.733	17.273	17.286	17.400	17.111
Spinous Rays	1.033	0.550	0.488	0.894	0.751
Dorsal	8.133	8.091	8.143	9.600	9.037
Soft Rays	0.834	0.868	1.215	1.140	1.224
Anal Fin Rays	9.867	9.909	10.000	10.600	10.037
	0.915	0.294	0.000	0.894	1.018
Upper Tooth	1.933	1.636	1.000	1.000	1.074
Rows	0.258	0.492	0.000	0.000	0.267
Lower Tooth	2.067	2.182	2.000	2.000	0.037
Rows	0.458	0.733	0.000	0.000	0.338
Bilateral (Kurtosis ± SEK)					
Pectoral	-1.348	3.498	0.000	0.000	27.000
Fin Rays (L-R)	1.121	0.953	0.000	0.000	0.872*
Flank Bars	0.000	3.168	0.000	0.000	7.353
(L-R)	0.000	0.953	0.000	0.000	0.872*
Pelvic	0.000	0.000	0.000	0.000	0.000
Fin Rays (L-R)	0.000	0.000	0.000	0.000	0.000

Table 4. Principal components generated from meristic data and the percentage of variation explained by each for parental individuals of four *Metriaclima* species. The characters with the highest factor loadings for each principal component are listed.

Principal Component	% Variance	Character	Loadings
PC I	60.607	Dorsal-fin rays	0.958
		Pectoral fin rays (L)	0.867
		Pectoral fin rays (R)	0.732
		Teeth rows (lower)	0.861
		Flank Bars (right)	-0.982
		Flank Bars (left)	-0.997
PC II	27.116	Anal fin	0.962
		Dorsal-fin spines	0.655
PC III	12.277	Teeth rows (upper)	0.874

Table 5. Principal components generated from Stauffer *et al.* (1997) morphometric and meristic data, and the percentage of variation explained by each for *M. thapsinogen*, *M. emmiltos*, *M. zebra* (Chitande), *M. zebra* (Namalenje), and *M. zebra* (Nkhata Bay). Characters with the high factor loadings are listed for each principal component.

Principal Component	% Variance	Character	Loadings
Morphometrics			
PC I	57.271	Standard length	-0.960
		Head length (mm)	-0.835
		Snout to dorsal-fin origin	0.922
		Snout to pelvic-fin origin	-0.844
		Dorsal-fin base length	0.826
		Anterior dorsal to anterior anal	0.948
		Posterior anal to dorsal caudal	0.836
		Posterior dorsal to ventral caudal	0.817
		Posterior dorsal to pelvic-fin origin	0.851
		Horizontal eye diameter	0.971
		Vertical eye diameter	0.975
		Postorbital head length	-0.817
PC II	25.052	Head length (% SL)	0.830
		Pelvic-fin length	0.754
		Anterior dorsal to pelvic-fin origin	0.734
		Snout length	0.931
Meristics			
PC I	44.400	Scale rows on cheek	0.746
		Anal-fin rays	0.982
		Pectoral-fin rays	0.982
		Gill rakers on first ceratobranchial	-0.765
PC II	29.626	Pored scales posterior to lateral line	0.821
		Teeth in outer row of left lower jaw	-0.960

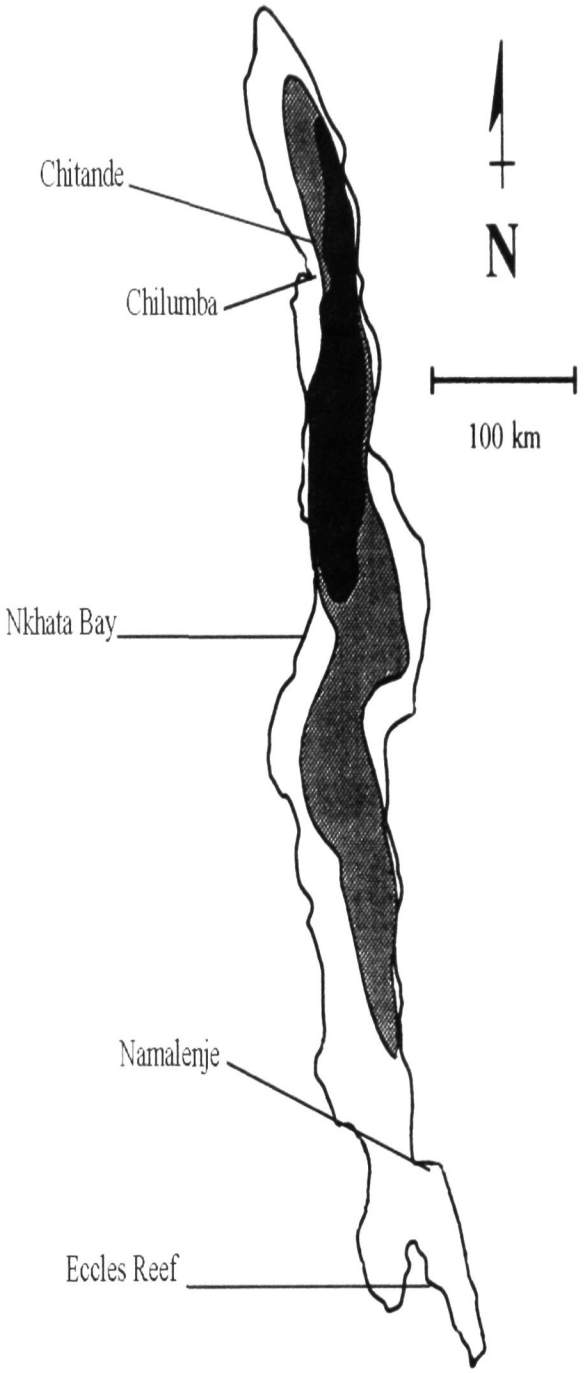

Figure 1 Map of Lake Malawi showing collection localities of *M. emmiltos* (Chilumba), *M. zebra* (Chitande), *M. zebra* (Nkhata Bay), *M. zebra* (Namalenje), and *M. thapsinogen* (Eccles Reef).

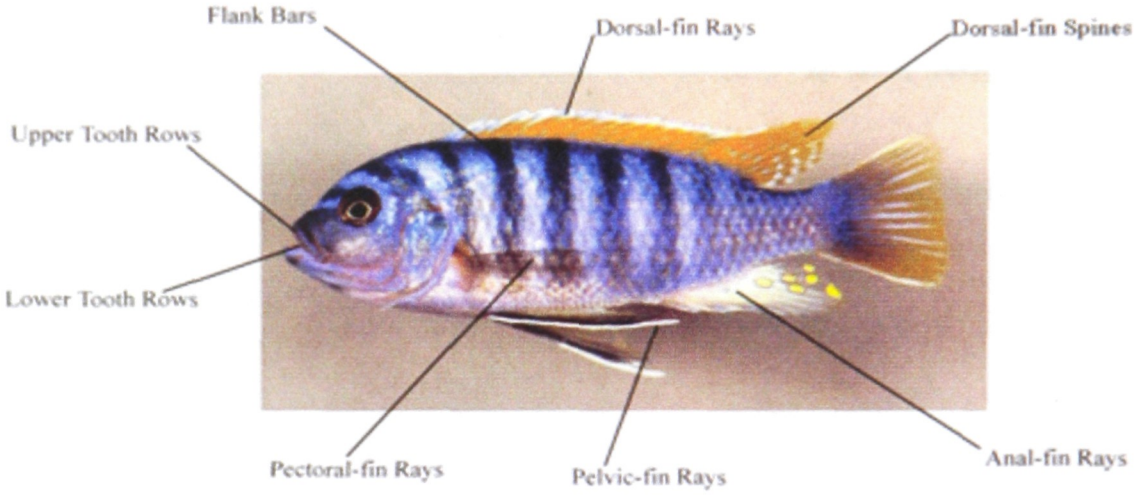

Figure 2 *Metriaclima pyrsonotos* from Nakantenga with eight meristic characters assessed in parental individuals of *M. thapsinogen*, *M. emmiltos*, *M. zebra* (Namalenje), *M. zebra* (Nkhata Bay) and F1 hybrid individulas.

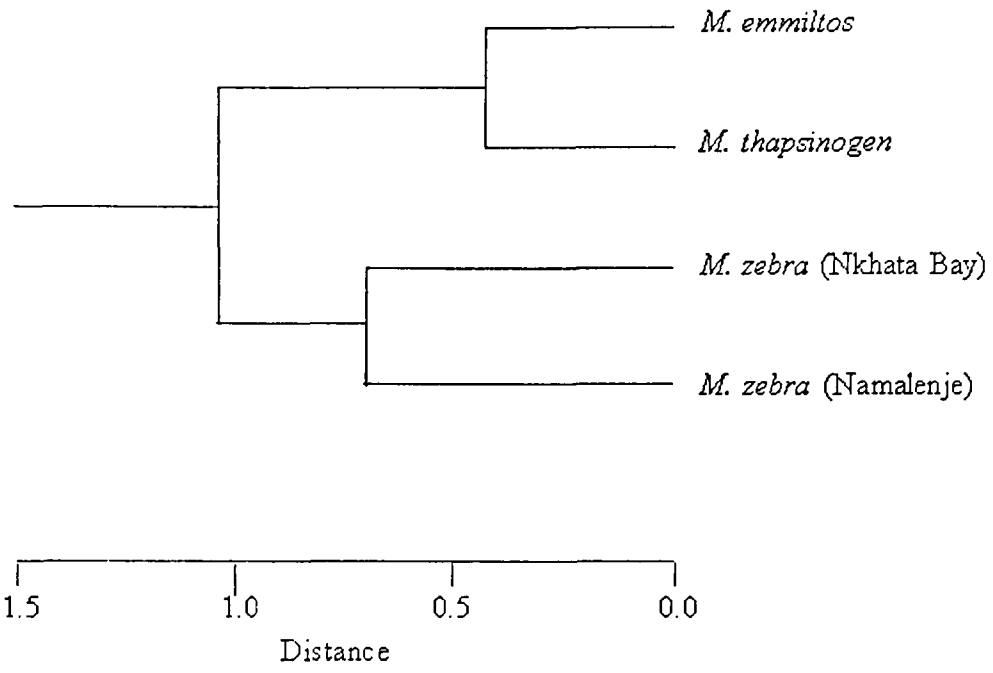

Figure 3 UPGMA clustering of relationships among *M. emmiltos*, *M. thapsinogen*, and *M. zebra* (northern and southern) populations. The red dorsal populations clustered more closely together than they did to their geographically proximate blue-black populations.

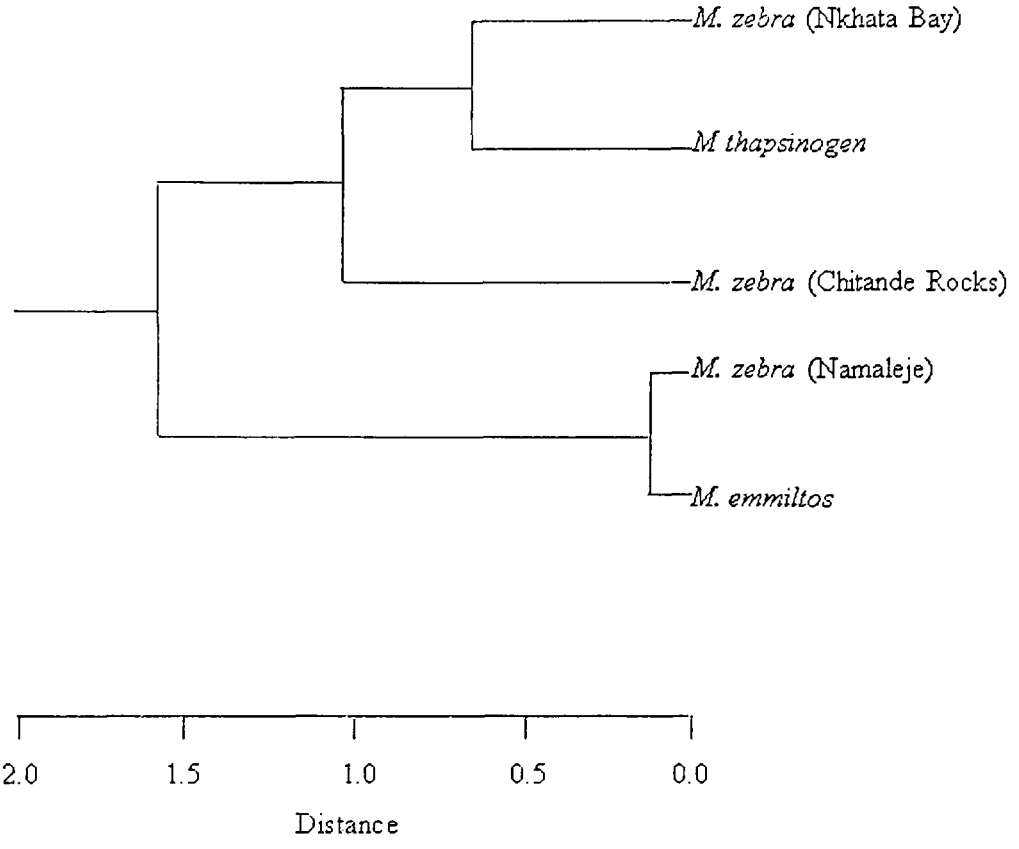

Figure 4 UPGMA clustering of Stauffer *et al.* (1997) morphometric and meristic data. Groupings represent relationships among Lake Malawi cichlid populations. Geographically similar populations clustered more closely together.

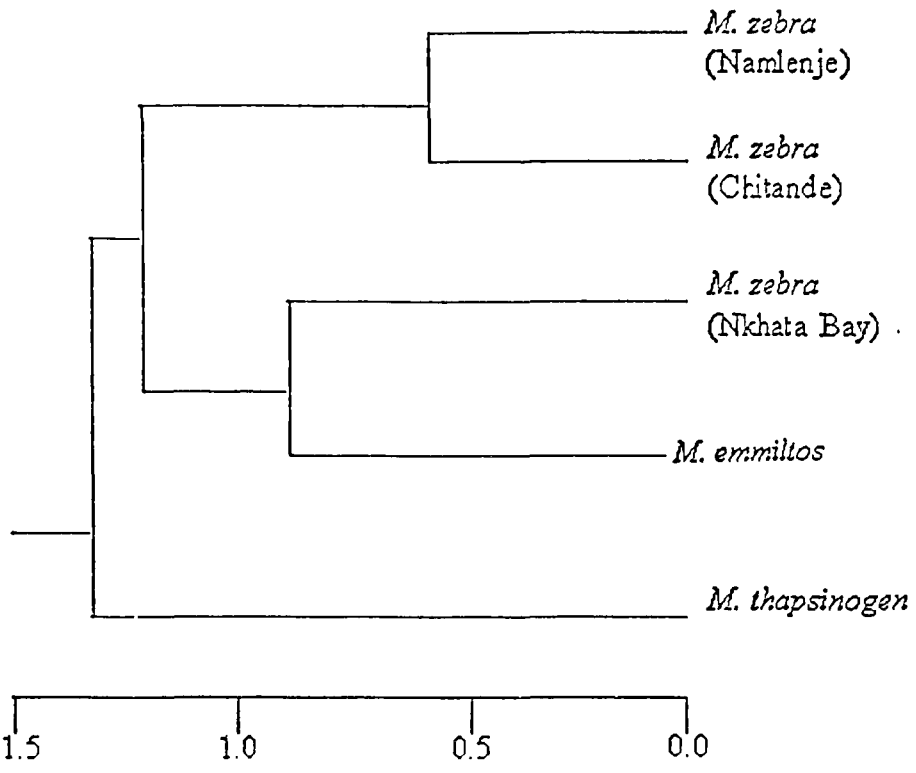

Figure 5 UPGMA of Stauffer *et al.* (1997) meristic data.

LITERATURE CITED

Albertson, R. C., Markert, J. A., Danley, P. D., and Kocher, T. D. 1999. Phylogeny of a rapidly evolving clade: The cichlid fishes of Lake Malawi, East Africa. Proceedings of the National Academy of Science USA 96: 5107-5110.

Burnaby, T. P. 1966. Growth-invariant discriminant functions and generalized distances. Biometrics 22: 96-110.

Crossley, R. Davison-Hirschmann S., Owen R. B., Shaw P. 1984. Lake level fluctuations during the last 2000 years in Malawi. pp. 305 - 316. *In*: Late Cainozoic Palaeoclimates of the southern hemisphere (J. C. Vogel ed.). Rotterdam: A.A. Balkema.

Deutsch, James C. 1997. Colour diversification in Malawi cichlids: evidence for adaptation, reinforcement or sexual selection? Biological Journal of the Linnean Society 62: 1-14.

Eccles, D. H., and Trewavas, E. 1989. Malawian Cichlid Fishes: The Classification of some Haplochromine Genera. Lake Fish Movies, Herten, Germany.

Fryer, G. 1959. The trophic interrelationships and ecology of some littoral communities of Lake Nyasa with especial reference to the fishes, and a discussion of the evolution of a group of rock-frequenting Cichlidae. Proceeding from the Zoological Society of London 132: 153-281.

Fryer, G. 1977. Evolution of species-flocks of cichlid fishes in African Lakes. Zeitschrift für zoologische systamatik und Evolutionsforschung 15: 141-165.

Fryer G. and Iles T. D.1972. The cichlid fishes of the Great Lakes of Africa: Their Biology and Evolution. Oliver and Boyd, Edinburgh, Scotland.

Grant, P. R. 1986. Ecology and Evolution of Darwin's Finches. Princeton University Press, Princeton, NJ.

Greenwood, P. H. 1991. Speciation. pp. 86-102. *In*: Cichlids Fishes: Behaviour, Ecology and Evolution (M. Keenleyside ed.). Chapman and Hall, London, UK.

Grobler, P. J. and Taylor, P. J. 1999. Fluctuating asymmetry and allozyme variability in an isolated springbok *Antidorcas marsupialis* population from the Chelmsford Nature Reserve. Acta Theriologica 44(2): 183-193.

Guthrie, D.M. and Muntz, W.R.A. 1993. Role of Vision in Fish Behavior. pp 89-128. *In*: Behaviour of Teleost Fishes (T.J. Pitche 2nd edition). Chapman and Hall, London, UK.

Hori, M. 1993. Frequency-dependent natural selection in the handedness of scale-eating cichlid fish. Science 260: 216-219.

Houde, A.E. and Endler, J. A.1990. Correlated evolution of female mating preferences and male color patterns in the guppy *Poecilia reticulata*. Science 248: 1405 - 1408.

Klingenberg, C. P. and Nijhout, H. F. 1999. Genetics of fluctuating asymmetry: A developmental model of developmental instability. Evolution 53(2): 358-375.

Konings, A. 1990. Cichlids of Lake Malawi. TFH Publications, Neptune City, N.J.

Kornfield, I. 1978. Evidence of rapid speciation in African cichlid fishes. Experientia 34: 335-336.

Kornfield, I. 1991. Genetics. pp. 103-128. *In*: Cichlid fishes Behaviour, Ecology and Evolution (M. Keenleyside ed.). Chapman and Hall, London, UK.

Kornfield, I and Parker, A. 1997. Molecular systematics of a rapidly evolving species flock: The mbuna of Lake Malawi and the search for phylogenetic signal. pp. 25-37. *In*: Molecular Systematics of Fishes. (T. D. Kocher and C. A. Stepien eds.). Academic Press, San Diego, CA.

Lewis, D. S. C., Reinthal, P., and Trendall, J. 1986. A guide to the fishes of Lake Malawi National Park. World Wildlife Fund. Gland, Switzerland.

Mayer, W.E., Tichy, H., and Klein, J. 1998. Phylogeny of African cichlid fishes as revealed by molecular markers. Heredity 80: 702-714.

McElroy, D. H., Kornfield, I., Everett, J. 1991. Coloration in African cichlids: diversity and constraints in Lake Malawi endemics. Netherlands Journal of Zoology 41: 250-268.

McKaye, K. R. and Gray, W. M. 1984. Extrinsic barriers to gene flow in flock-dwelling cichlids of Lake Malawi: macrohabitat heterogeneity and reef colonization. pp. 169-183. *In:* Evolution of Fish Species Flocks (A.A. Echelle and I. Kornfield eds.). University of Maine at Orono Press, Orono, ME.

Meyer, A., Kocher, T. D., Basasibwaki, P. and Wilson, A. C. 1990. Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. Nature 347: 550-553.

Moran, P and Kornfield, I. 1993. Retention of an ancestral polymorphism in the mbuna species flock (Pisces: Cichlidae) of Lake Malawi. Molecular Biology and Evolution 10: 1015-1029.

Owen, R. B., Crossley, R., Johnson, T. C., Tweedle, D., Kornfield, I., Davison, S., Eccles, D. H., Engstrom, D. E. 1990. Major low levels of Lake Malawi and their implication for speciation rates in cichlid fishes. Proceedings of the Royal Society, London, Biological Sciences 240: 519-553.

Reinthal, P. 1987. Morphology, ecology and behaviour of groups of the rock-dwelling cichlid fishes (Cichlidae: Perciformes) from Lake Malawi, Africa, Ph.D. thesis. Yale University.

Ribbink, A. J. 1991. Distribution and ecology of the cichlids of the African Great Lakes. pp. 36-59. *In*: Cichlid Fishes: Behavior, Ecology and Evolution (M. Keenleyside ed.). Chapman and Hall, London, UK.

Ribbink, A. J., Marsh B. A., Marsh, A. C., Ribbink, A. C., Sharp, B. J. 1983a. A preliminary survey of the cichlid fishes of rocky habitats in Lake Malawi. South African Journal of Zoology. 18: 149-310.

Rohlf, F. J. 2000. NTSYS-pc, Version 2.10. Exeter Software, Setauket, NY.

Scholtz, C. A., Rosendahl, B. R. 1988. Low lake stands in Lakes Malawi and Tanganyika, East Africa, delineated with manifold seismic data. Science 240: 1645-1648.

Seehausen, O., van Alphen , J. J. M. and Witte F. 1999. Can ancient polymorphisms explain why some cichlid lineages speciate rapidly under disruptive sexual selection? Belgian Journal of Zoology 129: 43-60.

Seehausen, O. and van Alphen, J .J. M. 1998. The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex). Behavioral Ecology and Sociobiology 42: 1-8.

SPSS, Inc. 1999. SYSTAT 9.0. SPSS Inc. Chicago, IL.

Stauffer, J. R. Jr. 1994. A new species of *Iodotropheus* (Teleostei: Cichlidae) from Lake Malawi, African Ichthyological Explorations of Freshwater 5: 331-344.

Stauffer, J. R. Jr., Bowers, N.J., Kellogg, K. A., and McKaye, K. R. 1997. A revision of the blue-black *Pseudotropheus zebra* (Teleostei: Cichlidae) complex from Lake Malawi, Africa, with a description of a new genus and ten new species. Proceedings from the Academy of Natural Sciences of Philadelphia 148: 189-230.

Stiassny, M. L. J. 1991. Phylogenetic interrelationships of the family Cichlidae: An overview. pp. 1-35. *In*: Cichlid Fishes Behaviour, Ecology and Evolution (M. Keenleyside ed.). Chapman and Hall, London, UK.

Trewavas, E. 1935. A synopsis of the cichlid fishes of Lake Nyasa. Annals and Magazine of Natural History, Ser10, 16: 65-118.

Turner, G. F. 1996. Offshore Cichlids of Lake Malawi. Cichlid Press, St. Leon Rot, Germany.

Woods, R. E., Sgrò, C. M., Hercus, M. J., and Hoffmann, A. A. 1999. The Association between fluctuating asymmetry, trait variability, trait heritability, and stress: A multiply replicated experiment on combined stresses in *Drosophila melanogaster*. Evolution 53 (2): 493-505